## REMARKS

Reconsideration of the above-identified application in view of the amendment above and the remarks below is respectfully requested.

No claims have been canceled or added in this paper. Claims 1-12, 14-18, 20-23, 25-26, 30-34, 36 and 41 have been amended in this paper. Therefore, claims 1-12, 14-18, 20-23, 25-26, 30-34, 36 and 41 are pending and are under active consideration.

Claims 1-12, 14-18, 20-23, 25-26, 30-34, 36 (it is believed that the reference to claim 35 should be to claim 36) and 41 stand objected to for the following reasons:

> Claims 2-12, 14-18, 20-23, 25, 26, 30-34, 35, and 41 do not begin with an article which is required by rules of grammar, and further required to ascertain if a dependent claim requires all limitations of the claim from which it depends (when the article is "the") or if the dependent claim only requires a portion of the limitations of the claim from which it depends (when the article is "a").
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> Claims 1-12, 14-18, 20-23, 25, 26, 30-34, 35, and 41 recite biological samples that should be modified by an adjective in claim 1, but instead are modified by a noun "cancer" in the wherein clause. The term "cancer" should be amended to recite "cancerous."

With respect to the first ground recited above in support of the objection, Applicants have amended the claims in question so that all now begin with an article. With respect to the second ground recited above in support of the objection, Applicants have adopted the Patent Office's suggestion to amend claim 1 to recite "cancerous," instead of "cancer."

Accordingly, the subject objection has been obviated and should be withdrawn.

Claims 1-12, 14-18, 20-23, 25, 26, 30-34, 36 (it is believed that the reference to claim 35 should be to claim 36) and 41 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claims 1-12, 14-18, 20-23, 25, 26, 30-34, 35, and 41 are indefinite for recitation of the phrase "wherein said groups are either healthy and cancer or cancer" because the nature of the groups is not clearly defined. It is not clear if there are two or three groups, or if one group is healthy and cancerous. It is further unclear how a group can be both healthy and cancerous if there are only two groups listed. It is further unclear how at least two groups can be selected if the claim is limited to only two groups.

Applicants respectfully traverse the subject rejection. Claim 1 has been amended herein so that the language in question now recites "wherein each of said groups is cancerous." Applicants respectfully submit that this language is clear and overcomes the rejection.

Accordingly, the subject rejection should be withdrawn.

Claims 1-8, 10-11, 14-15, 17-18, 20, 22, 23, 25-26, 30-34 and 41 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to a method of determining differential gene expression between normal and cancerous tissue samples, analyzing methylation states of the gene, wherein the methylation state is determined by use of a bisulfite reaction, and adding identified differentially expressed and methylated genes to a database.
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> Kikyo et al. shows in the abstract and throughout a method of analysis of mouse embryo tissue for genes that are differentially

expressed between normal embryos and abnormal embryos with chromosomal translocations. A differentially expressed neuronatin (Nnat) gene was shown to be imprinted by methylation analysis. Kikyo et al. shows on pages 68-69 differential display analysis of mRNA from the embryos, in which eighty primer pairs were used, and approximately 80-100 bands per primer pair were observed. Ten differentially expressed bands corresponding to differentially expressed genes were observed. Two genes were identified as H19 and Nnat (see figures 1A and 1B). Kikyo et al. further noted on page 69 prior art that used subtraction hybridization to identify Nnat as a differentially expressed gene, and verified Nnat differential expression by a reverse transcriptase-polymerase chain reaction method (see figure 1C). Kikyo et al. subsequently analyzed the Nnat gene for methylation by digestion with a panel of restriction endonucleases Hind III, BssH II, Eag I, and Sac II (see figure6).

The New England Biolab website establishes that BssH II, Eag I and Sac II enzymes are inhibited by methylation at CpG sites.

Siegfried et al. establishes on page R305 that CpG methylation is a term of art meaning that a cytosine is methylated.

Kikyo does not show use of a bisulfite reaction to determine methylation states of cytosine or analysis of tissues from normal and cancerous samples.

Frommer et al. states on page 1827 that cytosine methylation has long been recognized as an important factor in the silencing of genes in mammalian cells. Frommer et al. shows in the abstract and throughout a method to determine the positions of methylated cytosine residues in DNA by use of sodium bisulfite to convert cytosine to uracil in a chemical reaction (which does not react with methylated cytosine). Frommer et al. shows in page 1828 and figure 1 that their method comprises polymerase chain reactions subsequent to the sodium bisulfite treatment that produces polynucleotides suitable for sequencing reaction analysis. The sequence analysis of the amplified products reveals the presence of positions that originally contained methylated cytosine (see figures 2 and 3). Frommer et al. lists advantages of their method on page 1830, including the positive display of methylated cytosine residues, and the capacity to analyze individual strands of a DNA sample.

> Huang et al. shows in the abstract that CpG methylation is known to be associated with gene silencing in cancer, and reviews the prior art showing the relationship between cancer and methylation states of DNA on page 459. Huang et al. shows analysis of methylation states in CpG regions in cancerous versus normal controls from cell lines in figures 3,4,5, and 7, and primary breast tissue versus normal breast tissue in figures 6 and 8. Huang et al. shows a database of CpG clones that exhibit altered methylation in Table 1.
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> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. by use of the sodium bisulfite reaction method of methylated cytosine detection of Frommer et al. because Frommer et al. shows that their method also detects methylated cytosine, and has further advantages of positive display of methylated cytosine residues and the ability to analyze individual stands of a DNA sample. It would have been further obvious to use samples of normal and cancerous tissue because Huang et al and Frommer et al. show that metylation regulates gene expression in mammalian cells and because Huang et al. shows that methylation is associated with gene silencing in cancer, and because Huang et al. shows analysis of CpG clones from normal and cancerous cell lines and primary clinical samples for differential methylation. It would have been further obvious to generate a database of the results of the method for the purpose of retaining the results for later review, as suggested by Huang et al. in table 1.

Applicants respectfully traverse the subject rejection. As noted above, claim 1, from which the remaining rejected claims depend, has been amended herein to recite, amongst other things, that each of the at least two groups of biological material containing mRNA and/or proteins is cancerous. The applied references, whether taken individually or in combination, do not teach or suggest that each of at least two groups of biological material containing mRNA and/or proteins is cancerous.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1, 6, 9, 16, 21 and 36 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above and further in view of Danssaert et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to the method of claim 1 with the further limitation that the methylation analysis comprises use of a robot or a computer device.
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> Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above does not show a methylation analysis that comprises use of a robot or a computer device.
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> Danssaert et al. shows in column 1, lines 22-25 that polymerase chain reactions are best performed on automated devices that allow for consistent thermal cycling. Danssaert et al. shows computer controlled thermal cyclers that comprise robotic arms in column 1, line 33, column 4, and lines 39-50, column 5, lines 31-48.
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> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above by use of a computer controlled thermal cycler, optionally with robotic arms, for conducting the polymerase chain reactions because Danssaert et al. shows that automated thermal cyclers have the advantage of providing consistent thermal cycling, and further because it is obvious to automate a manual activity (see MPEP 2144.04).

Applicants respectfully traverse the subject rejection. Claim 1 is patentable over Kikyo et al., New England Biolabs, Siegfried et al., Frommer et al. and Huang et al. for at least the reasons above. Danssaert et al. fails to cure all of the deficiencies of these references. Therefore, claim 1 is patentable over the applied combination of references. Claims 6, 9, 16, 21 and 36 depend from claim 1 and are patentable over the applied combination of references based at least on their respective dependencies from claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1 and 12 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above, and further in view of Anderson et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to the method of claim 1 with the further limitation that both mRNA and protein levels are measured.
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> Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above does not show measurement of protein levels.
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> Anderson et al. shows comparison of human liver gene expression by measurement of mRNA levels and corresponding protein levels (as measured by two-dimensional protein electrophoresis). Anderson et al. shows moderate levels of correlation between mRNA levels and protein levels in figures 1 and 2. Anderson et al. conclude on page 537 that determination of protein levels allows for a better understanding of multi-level gene expression control in complex organisms such as man.

> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. in view of Huang et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above by additional use of the protein analysis method of Anderson et al. because Anderson et al. shows that determination of correlations between mRNA and protein levels allows for better understanding of gene expression controls.

Applicants respectfully traverse the subject rejection. Claim 1 is patentable over Kikyo et al., New England Biolabs, Siegfried et al., Frommer et al. and Huang et al. for at least the reasons above. Anderson et al. fails to cure all of the deficiencies of these references. Therefore, claim 1 is patentable over the applied combination of references. Claim 12 depends from claim 1 and is patentable over the applied combination of references based at least on its dependency from claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on October 19 2007.

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